Infection, Genetics and Evolution 43 (2016) 146–150

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

Heterozygous mutants of *TIRAP* (S180L) polymorphism protect adult patients with *Plasmodium falciparum* infection against severe disease and mortality

Aditya K. Panda [a,b], Bidyut K. Das [b,c,*], Abhinash Panda [c], Rina Tripathy [d], Sarit S. Pattnaik [c], Harishankar Mahto [a], Sylviane Pied [e], Sulabha Pathak [f], Shobhona Sharma [f], Balachandran Ravindran [b,**]

[a] Centre for Life Sciences, Central University of Jharkhand, Brambe, Ranchi, India
[b] Infectious Disease Biology Group, Institute of Life Sciences, Bhubaneswar, Odisha, India
[c] Department of Medicine, S.C.B. Medical College, Cuttack 753007, Odisha, India
[d] Department of Biochemistry, S.C.B. Medical College, Cuttack 753007, Odisha, India
[e] Basic and Clinical Immunology of Parasitic Diseases Group, Centre for Infection and Immunity of Lille, 59019 Lille Cedex, France
[f] Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai 400005, India

ARTICLE INFO

Article history:
Received 31 December 2015
Received in revised form 19 April 2016
Accepted 28 April 2016
Available online 7 May 2016

Keywords:
TIRAP
Polymorphism
TNF-α
Meta-analysis
P. falciparum malaria

ABSTRACT

Toll-interleukin-1 receptor domain containing adapter protein (TIRAP) plays a crucial role in TLR2 and TLR4 signaling pathways. Glycosylphospatidylinositol (GPI), considered a toxin molecule of *Plasmodium falciparum*, interacts with TLR2 and 4 to induce an immune inflammatory response. A single nucleotide polymorphism at coding region of *TIRAP* (S180L) has been reported to influence TLRs signaling. In the present study, we investigated the association of *TIRAP* (S180L) polymorphism with susceptibility/resistance to severe *P. falciparum* malaria in a cohort of adult patients from India. *TIRAP* S180L polymorphism was typed in 347 cases of severe malaria (SM), 232 uncomplicated malaria and 150 healthy controls. Plasma levels of TNF-α was quantified by ELISA. Heterozygous mutation (S/L) conferred significant protection against MOD (multi organ dysfunction), NCSM (non-cerebral severe malaria) as well as mortality. Interestingly, homozygous mutants (L/L) had 16 fold higher susceptibility to death. TIRAP mutants (S/L and L/L) were associated with significantly higher plasma TNF-α levels compared to wild type (S/S). The results of the present study demonstrate that *TIRAP* S180L heterozygous mutation may protect patients against severe malaria and mortality.

© 2016 Elsevier B.V. All rights reserved.

1. Background

Malaria is caused by infection of various species of genus *Plasmodium* (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium knowlesi*) and a recent report by World Health Organization (WHO) projected about 198 million cases worldwide in the year 2013 (WHO, 2014). Although the report highlighted reduction of mortality rate worldwide, *P. falciparum* infection still remains the major cause of death due to malaria (WHO, 2014). *P. falciparum* infected patients show a wide range of clinical manifestations such as a) cerebral malaria, b) multi organ dysfunction, c) non-cerebral severe malaria and d) uncomplicated malaria (Panda et al., 2011, 2012). The exact cause of such clinical heterogeneity is not known, however, it has been attributed to parasite strain virulence difference, host immune response and secondary bacterial infections (Miller et al., 2002).

Host immune response, particularly the role of Toll like receptors (TLRs) has been widely investigated in malaria. Glycosylphospatidylinositol (GPI), a 'toxin' of *P. falciparum* has been identified as an important factor that activates host innate immune system through TLR2 and TLR4 (Gowda, 2007). Activation of the TLRs system elicits production of proinflammatory molecules such as tumour necrosis factor (TNF)-α, interleukin-1β (IL-1β) and IL-12 which has been proposed to facilitate elimination of parasites (Malaguarnera and Musumeci, 2002; Walther et al., 2006). An adapter molecule, Toll-interleukin-1 receptor domain containing adapter protein (TIRAP), also known as MAL (MYD88 adapter like) is essential for both TLR2 and TLR4 signaling (Fitzgerald et al., 2001; Yamamoto et al., 2002). A common functional polymorphism at coding region (S180L) has been reported in the TIRAP gene (NP_683708.1:p.Ser180Leu), which enhances this activation process (Akira and Takeda, 2004; Ferwerda

* Correspondence to: B.K. Das, Department of Medicine, SCB Medical College, Cuttack, Odisha, India.
** Correspondence to: B. Ravindran, Infectious Disease Biology Group, Institute of Life Sciences, Bhubaneswar 751023, Odisha, India.
E-mail addresses: adityarmrc@gmail.com (A.K. Panda), bidyutdas@hotmail.com (B.K. Das), drnashville@gmail.com (A. Panda), rinatripathy@hotmail.com (R. Tripathy), saritsekhar@gmail.com (S.S. Pattnaik), sylviane.pied@pastur-lille.fr (S. Pied), pathaksue@gmail.com (S. Pathak), shobhona@gmail.com (S. Sharma), ravindran8@gmail.com (B. Ravindran).

http://dx.doi.org/10.1016/j.meegid.2016.04.035
1567-1348/© 2016 Elsevier B.V. All rights reserved.

et al., 2009; Khor et al., 2007; Yamamoto et al., 2002). This polymorphism has been associated with protection from several infectious diseases such as tuberculosis, bacterial septicemia, invasive pneumococcal disease (Jenkins and Mansell, 2010), and autoimmune diseases like systemic lupus erythematosus and Behcet's disease (Durrani et al., 2011). Association of *TIRAP* (S180L) polymorphism with outcome in *P. falciparum* malaria has been reported (Esposito et al., 2012; Hamann et al., 2009; Khor et al., 2007; Zakeri et al., 2011), but studies in the context of severe *P. falciparum* infection are limited (Khor et al., 2007).

The current study was designed to evaluate the association of the TIRAP S180L polymorphism with susceptibility/resistance to severe *P. falciparum* malaria in adults in an endemic area of Odisha, India. The role of the *TIRAP* S180L polymorphism on plasma TNF-α levels was also investigated in the present study.

2. Materials and methods

2.1. Study site and sample collection

Patients attending and/or admitted to Department of Medicine, SCB Medical College and Hospital, Cuttack, Odisha during the year 2008–2012 were included in present study. The state of Odisha is considered endemic for malaria with >85% cases attributed to *P. falciparum* infection (NVBDCP, 2004). All patients enrolled in the current study came from the coastal districts (Cuttack, Khordha, Puri, Nayagarh, Angul, Dhenkanal) having an average annual parasite index (API) of 6.67 (NVBDCP, 2010). Malaria endemic areas in Odisha have a stable population dynamic which can be traced to several generations. Laboratory diagnosis of malarial infection was conducted by an immunochromatography test (ICT) (SD Bio Standard Diagnostics India) and nested PCR assay (Panda et al., 2011, 2012). Based on WHO guidelines and as described earlier (Panda et al., 2011, 2012; Pattanaik et al., 2012), patients were categorized into two broad groups: 1) uncomplicated malaria (UM) and 2) severe malaria (SM). Severe malaria (SM) cases were further categorized into three sub-groups i) cerebral malaria (CM) defined as patients with altered sensorium, GCS (Glasgow Coma Scale) of ≤10; ii) non cerebral severe malaria (NCSM) patients had one of the several manifestations of severe malaria without cerebral involvement, namely severe anaemia (haemoglobin <5 g/dl), acute renal failure (serum creatinine >3 mg/dl), jaundice (serum bilirubin >3 mg/dl), acute respiratory distress syndrome (PaO2/FIO2 < 200), haemoglobinuria (dark red or black coloured urine positive for haemoglobin) and shock (systolic BP of <80 mm Hg); and iii) multi-organ-dysfunction (MOD) was diagnosed based on the presence of two or more organ involvement like CNS (GCS ≤ 10), respiratory (PaO2/FIO2 < 200), renal failure (serum creatinine >3 mg/dl) and hepatic dysfunction (ALT/AST > 3 times of normal, prolonged prothrombin time and albuminaemia). Patients with the following criteria were excluded from the current study: i) co-infection with other *Plasmodium* species, ii) chronic diseases like tuberculosis, chronic renal failure, liver cirrhosis and autoimmune diseases like systemic lupus erythematosus and rheumatoid arthritis. 150 healthy controls (HC) from a similar geographical background (coastal districts) unrelated to each other were enrolled. None of the healthy controls reported history of clinical malaria in the last 5 years and were free of *P. falciparum* infection investigated by ICT and nested PCR (Panda et al., 2011, 2012). The risk of exposure to malaria was similar for both HC and patients since they came from similar geographical areas. About 5 ml of venous blood was collected in EDTA vials from all enrolled patients. The study and its protocol were approved by the Institutional Human Ethics Committee of S.C.B. Medical College Cuttack and Institute of Life Sciences, Bhubaneswar. Blood samples were collected after obtaining written consent of the healthy controls and patients or accompanying person (in case of comatose patients).

2.2. DNA isolation and genotyping of TIRAP (S180L) polymorphism

DNA was isolated from whole blood by Gen Elute Blood Genomic DNA Kit (Sigma-Aldrich). Samples were genotyped for *TIRAP* (S180L) polymorphism by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) as described earlier (Khor et al., 2007). In brief, primers (TIRAP-S/L-F: CTCCAGGGGCCGAGGGCTGCAC CATCCCC[C → A]TGCTG; TIRAP-S/L-R: TACTGTAGCTGAATCCCGTTCC) were obtained from Integrated DNA Technologies Coralville, Iowa. PCR was carried out in following reaction condition which gives an amplicon size of 193 bp: initial denaturation of 95 °C for 5 min, 35 cycle of 95 °C for 30 s, 52 °C for 30 s, 72 °C for 30 s and a final extension of 72 °C for 5 min. The PCR product was digested by *BstXI* (New England BioLabs). RFLP products were separated on 3.5% neusive agarose and analyzed under UV illumination. For confirmation of genotyping results, about 20% of the randomly selected samples were directly sequenced and 100% concordance was observed.

2.3. TNF-α quantification

The plasma TNF-α was quantified by enzyme linked immunosorbent assay (ELISA) kit (eBiosciences) according to manufacturer's instructions.

2.4. Statistical analysis

Genotype and allele frequency were calculated by direct counting. SNPalyze software (Dynacom, Japan) was employed to calculate Hardy-Weinberg equilibrium. Fisher's test was used for comparison of genotype, allele frequencies with clinical data. Odds ratios (ORs), 95% confidence intervals (95% CIs) were calculated by Graphpad prism 5.01. For analysis of data shown in Table 1, the allele and genotypes with higher frequency were selected as reference (OR = 1) and the other ORs were calculated relative to that reference (Fisher's exact test, 2 × 2 contingency tables). P value < 0.01 (0.05/4: Bonferroni correction for four clinical categories) was taken as significant. Association of plasma TNF-α levels and TIRAP S180L polymorphism was assessed by analysis of variance (ANOVA) followed by Tukey multiple comparison posttest. Power analysis was performed using the statistical program G*Power 3.1 (Faul et al., 2007).

3. Results

3.1. Characteristics of study participants

A total of 579 *P. falciparum*-infected patients were enrolled in the current study, including 347 patients of severe malaria (SM) and 232 patients of uncomplicated malaria (UM). Severe *P. falciparum* malaria patients were further subdivided into cerebral malaria (CM) (n = 92), multi-organ dysfunction (MOD) (n = 136) and non-cerebral severe malaria (NCSM) (n = 119). A total of 150 healthy controls (HC) from similar geographical area were also included. The difference of mean age between all clinical categories (UM: 32.14, CM: 30.77, MOD: 32.09, NCSM: 33.02 years) and HC was comparable (31.24 years).

3.2. Prevalence of TIRAP S180L genotypes in control population

150 healthy controls were genotyped for TIRAP S180L polymorphisms. As shown in Table 1, the prevalence of S/S and S/L genotypes of *TIRAP* polymorphism was 65% and 34% respectively. Distribution of TIRAP S180L polymorphism was in Hardy-Weinberg equilibrium ($\chi^2 = 2.75$, P = 0.09). Two individuals were found to be homozygous mutant (L/L) type. The minor allele (L) frequency in healthy controls was 15%. A post hoc power of the study was calculated to detect the association between *TIRAP* S180L polymorphisms and severe *P. falciparum* malaria at 0.05 level of significance, assuming small effect size ($w =$

Download English Version:

https://daneshyari.com/en/article/2822951

Download Persian Version:

https://daneshyari.com/article/2822951

Daneshyari.com